bio-protocol Preprint

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DNA staining, flow cytometry, and confocal microscopy

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An abbreviated version of this protocol was published in eLIFE in Jan 2022

Stress conditions promote *Leishmania* hybridization in vitro marked by expression of the ancestral gamete fusogen HAP2 as revealed by single-cell RNA-seq

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Detailed protocol

Log-phase promastigotes were sedimented and resuspended in 1 mL PBS and fixed in 0.4% paraformaldehyde for 1 min at room temperature. Cells were sedimented, resuspended in 100 mL PBS and permeabilized with 1 mL methanol for 15 min on ice. Next, cells were sedimented, resuspended in 1 mL PBS and incubated for 10 min at room temperature. Staining was performed by adding 500 mL of PI-RNase mix (13 mg/mL each) to the cell suspension and incubating for 1 h at room temperature. Labeled cells were washed once in PBS and analyzed on a FacsCANTO II (BD Biosci-ences) flow cytometer. Hybrids ploidy was inferred by comparison with the DNA content of the parental lines.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sacks, D. (2022). DNA staining, flow cytometry, and confocal microscopy. Bio-protocol Preprint. bio-protocol.org/prep1917.
2. Louradour, I., Ferreira, T. R., Duge, E., Karunaweera, N., Paun, A. and Sacks, D.(2022). Stress conditions promote *Leishmania* hybridization in vitro marked by expression of the ancestral gamete fusogen HAP2 as revealed by single-cell RNA-seq. eLIFE. DOI: 10.7554/eLife.73488